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Regulation of interplay between autophagy and apoptosis in the diabetic heart

New role of AMPK

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Diabetes induces cardiomyocyte apoptosis and suppresses cardiac autophagy, indicating that the interplay between autophagy and apoptotic cell death pathways is important in the pathogenesis of diabetic cardiomyopathy. The potential mechanism, however, remains unknown. We recently reported that diabetes depresses AMP-activated protein kinase (AMPK) activity, inhibits MAPK8/JNK1-BCL2 signaling, and promotes the interaction between BECN1 and BCL2. Concomitantly, diabetes induces cardiomyocyte apoptosis and suppresses cardiac autophagy. Activation of AMPK directly phosphorylates MAPK8, which mediates BCL2 phosphorylation and subsequent BECN1-BCL2 dissociation, leading to restoration of cardiac autophagy, protection against cardiac apoptosis, and ultimately improvement in cardiac structure and function. We conclude that dissociation of BCL2 from BECN1 through activation of MAPK8-BCL2 signaling may be an important mechanism by which AMPK activation restores autophagy, protects against cardiac apoptosis, and prevents diabetic cardiomyopathy.

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Diabetic cardiomyopathy is characterized by ventricular dysfunction that occurs in diabetic patients independent of coronary artery disease, hypertension, or any other known cardiac disease. Diabetic cardiomyopathy has become a major cause of diabetes-related morbidity and mortality, and there is an urgent need to elucidate the mechanism of pathogenesis. We previously reported that in OVE26 mice, an established type 1 diabetic animal model, the activity of AMPK is reduced along with cardiac dysfunction and decreased cardiac autophagy. Chronic AMPK activation by metformin, a well-characterized AMPK activator, enhances autophagic activity, reduces apoptotic cell death, and preserves cardiac function, suggesting that regulation of the interaction between autophagy and apoptosis by AMPK is important in the pathogenesis of diabetic cardiomyopathy.

AMPK regulates many cellular processes, including energy metabolism, cell growth, apoptosis and autophagy. In the heart, AMPK is responsible for activation of glucose uptake and glycolysis during low-flow ischemia, and plays an important role in limiting apoptotic activity associated with ischemia and reperfusion. Activation of AMPK by metformin improves cardiac function and reduces the incidence of myocardial infarction in diabetic patients. In addition, AMPK has been identified as a positive regulator of autophagy in response to energy depletion and ischemic injury. AMPK was thought to regulate autophagy through inhibition of mammalian target of rapamycin complex 1, a negative regulator of autophagy, by either phosphorylation of tuberous sclerosis complex 2, which in turn deactivates the RHEB GTPase, or by phosphorylation of RPTOR. Recent studies suggest that AMPK directly stimulates autophagy through phosphorylation and activation of ULK1 [the mammalian homolog of yeast autophagy-related 1 (Atg1)], a

key initiator of the autophagic process. However, AMPK's involvement and the mechanism by which it regulates the switch between autophagy and apoptosis in the development of diabetic cardiomyopathy remain to be established.

Under diabetic conditions, cardiomyocyte apoptosis leads to decreased cardiac muscle mass, interstitial fibrosis and impaired cardiac function. Restoration of cardiac autophagy by AMPK activation is associated with amelioration of cardiomyopathy. We therefore investigated whether induction of autophagy serves as a protective response in the development of diabetic cardiomyopathy. In streptozotocin (STZ)-induced diabetic mice, impaired cardiac structure and function are accompanied by suppression of cardiac autophagy. Enhanced autophagy by metformin or overexpression of the ATG7 protein attenuates high glucose-induced apoptotic cell death, cardiac fibrosis and cardiac dysfunction, but these protective actions of metformin are abrogated by the autophagy inhibitor 3-methylademine and *Atg7* siRNA in cultured H9c2 cardiac myoblast cells. These data suggest that the induction of autophagy by AMPK activation constitutes a protective mechanism against cardiac structural and functional damage induced by diabetes.

We next investigated whether AMPK regulates the interaction of the autophagy protein BECN1 with anti-apoptotic protein B-cell CLL/lymphoma 2 (BCL2), a switch between autophagy and apoptosis, in the development of diabetic cardiomyopathy. BECN1 is a part of the class III phosphatidylinostol-3 kinase (PtdIns3K) lipid complex, which is required for initiation of autophagy. Binding of BCL2 to BECN1 inhibits BECN1-mediated autophagy via sequestration of BECN1 away from the class III PtdIns3K. We observed a strong interaction between BECN1 and BCL2 in H9c2 cells treated with elevated glucose levels and in hearts from diabetic animals. Treatment of cells or animals with metformin to activate AMPK results in disruption of the association between BECN1 and BCL2, and the free BECN1 being bound to the class III PtdIns3K to form a kinase complex, leading to initiation of autophagy.

Autophagy plays an essential role in cell growth, development and homeostasis. Constitutive autophagy helps to maintain a balance between the synthesis, degradation, and subsequent recycling of cellular components. It allows degradation of misfolded proteins that may be toxic to the cell, and removal of damaged organelles such as mitochondria to reduce oxidative stress and promote remodeling for survival. In addition, phosphorylated BCL2 could preserve the integrity of the mitochondrial outer membrane and prevent pro-apoptotic proteins from escaping (or being released) into the cytoplasm, thus protecting against apoptosis. Therefore, activation of AMPK prevents diabetes-suppressed autophagy and protects against apoptotic cell death through disruption of the BCL2 and BECN1 complex under diabetic conditions.

Our findings suggest that activation of MAPK8-BCL2 signaling is a new mechanism by which AMPK regulates autophagy. Our data indicate that activation of AMPK by metformin is associated with an increase in MAPK8 phosphorylation. In a cell-free system, recombinant AMPK dose-dependently increases MAPK8 phosphorylation. Moreover, exposure of H9c2 cells to a high-glucose environment, which inhibits MAPK8 phosphorylation, disrupts the association between AMPK and MAPK8. The disruption was depressed by metformin treatment, indicating that AMPK directly phosphorylates MAPK8. Since activation of MAPK8 results in phosphorylation of BCL2 and disruption of the BECN1-BCL2 complex under starvation conditions, we analyzed the role of AMPK-activated MAPK8 in regulating the interaction between BECN1 and BCL2 using gain- and loss-of-function approaches. Under normal and high-glucose conditions, the MAPK8 inhibitor SP600125 reduces MAPK8 phosphorylation. Co-immunoprecipitation experiments revealed that metformin reduces the association between BECN1 and BCL2 in normal glucose medium, whereas high glucose enhances the association between these two proteins and this interaction is disrupted by metformin treatment. Administration of SP600125 abrogates the effects of metformin on the BECN1-BCL2 complex, as BECN1 and BCL2 associate under these conditions. Correspondingly, metformin-enhanced autophagy is attenuated. Transfection of H9c2 cells with constitutively active MAPK8 plasmid disrupts the association between BECN1 and BCL2 and restores autophagic capacity under high-glucose conditions. Importantly, inhibition of MAPK8 by SP600125 abolishes metformin-reduced apoptotic cell death. Taken together, AMPK-activated MAPK8-BCL2 signaling is required for dissociation of BECN1 from BCL2, which stimulates autophagy and promotes cardiomyocyte survival.

In conclusion, activation of AMPK restores cardiac autophagy, protects against cardiac apoptosis, and ultimately improves cardiac structure and function through stimulation of MAPK8-BCL2 signaling and subsequent dissociation of BECN1 and BCL2. These findings provide new insights into the role of autophagy in the development of diabetic cardiomyopathy and deepen our understanding of how AMPK regulates autophagy. Definition of this mechanism will lead to new therapies toward diabetic cardiomyopathy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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